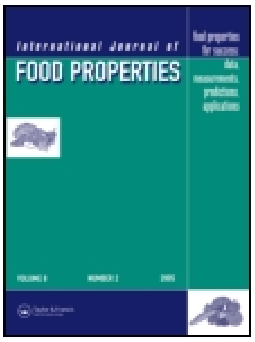


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# Isolation and Characterization of Starch from Pearl Millet (*Pennisetum typhoidium*) Flours

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Pearl millet, an underutilized crop, is a relatively good source of nutrients and has varied uses over cereals. New applications must be explored to popularize the millet. In the present study, flour and isolated starch from pearl millet cultivars—Kalukombu and Maharashtra Rabi Bajra (MRB)—were investigated for physicochemical properties, nutritionally important starch fractions and x-ray diffraction. The yield of starch was significantly low (Kalukombu: 34.5 g/100 g and MRB: 39.4 g/100 g) with traces of non-starch components (protein, fat, and ash) indicating its purity. Starch could be classified as non-waxy type based on low amylose content (2.86–4.96 g/100 g). Low amylose lead to fragility of swollen starch granules which disintegrated easily at 65°C as observed in swelling power and solubility. Isolated starch was characterized with low water- and oil-holding capacity which could be attributed to the low protein content and absence of fiber in the starch. However, MRB starch showed higher oil uptake compared to flour, possibly due to its larger surface area that increased oil uptake. X-ray diffraction patterns showed sharp peaks at 2θ values 15 and 23º and a diffused peak at 2θ of 17 and 18º which is characteristic of A-type pattern. Low resistant starch (RS) and high readily digestible starch (RDS) content observed in the isolated starch could be attributed to the A-type pattern of starch which is more susceptible to enzyme hydrolysis and also due to elimination of structural obstruction to amylase hydrolysis during the process of starch isolation and gelatinization.

*Keywords*: Nutritionally important starch fractions, Pearl millet, Starch isolation, X-ray diffraction.

## INTRODUCTION

Pearl millet is a multipurpose crop widely grown for food and non-food uses, such as feed, fodder, fuel, etc. It is a versatile millet mainly used in traditional food preparations, such as thick/thin porridges or Indian flat breads like chapatti/roti. Being gluten-free, pearl millet is suitable for subjects with celiac disease and has considerable potential as a novel food ingredient.[1,2] In terms of nutrient composition, pearl millet is comparable with commonly consumed cereals. Nevertheless, it is less popular and an underutilized crop. Hence, there is a decline in the food

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uses of pearl millet implying that its alternative use (non-food uses) has rapidly increased over the years.[2–4] In order to popularize the millet, new applications must be explored.

Starch, the major component of pearl millet is reported to range from 62.8 to 70.5 g/100 g in different genotypes. Commonly, starches are used in food products such as soup, stew, gravy, pie filling, sauce, or custard. It contributes greatly to the textural properties of foods and serves as a thickener, gelling agent, bulking agent, adhesive, etc.[5,6] Pearl millet being low-cost could be used as a relatively cheaper source of starch in the development of functional foods. Attempts to isolate starch from pearl millet have been previously reported.[7–9] Researchers have mostly investigated the composition and physicochemical properties of the isolated starch from pearl millet. However, information on the rate and extent of starch digestion is limited. Nutritionally, starch is divided into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). SDS is completely but slowly digested in the small intestine. RS is the starch fraction that escapes digestion in the small intestine. It reaches the large intestine, where it can be more or less fermented by the intestinal microflora.[10,11] This information is of great importance in formulating functional food products catering to target population.

In the present study, an attempt has been made to isolate starch from two certified pearl millet varieties Kalukombu (K) and Maharashtra Rabi Bajra (MRB), based on their availability and usage in Mysore city. K is a native pearl millet cultivar traditionally grown by farmers in India (Karnataka, Tamilnadu, and Maharashtra). This variety is not improved by the modern plant breeding system. It is considered nutritionally superior by the local people and is used as food crop to make traditional Indian preparations such as roti (unleavened bread), dumplings, and chapattis. The seeds of K are small and elongated with persisting glumes/husk (Fig. 1). MRB is a commercially grown hybrid developed by the modern improved plant breeding technique by a commercial seed company. It is basically a winter crop. The seeds are grey/slate colored, bold, and round shaped without persisting glumes/husk (Fig. 1).[12] The flours and isolated starch from the two pearl millet cultivars and corn starch (for comparison) were studied for physicochemical properties, x-ray diffraction, and nutritionally important starch fractions.

## MATERIALS AND METHODS

Certified varieties of pearl millet, namely K and MRB, (Fig. 1) were procured from the University of Mysore, Mysore, India.

**Kalukombu (K)** **Maharashtra Rabi Bajra (MRB)**

FIGURE 1 Pearl millet grains.

## Chemicals

Potato amylose (Type III) and corn was purchased from Himedia, Mumbai, India. The enzymes, such as Invertase, were purchased from Himedia, Mumbai, India; pancreatin from porcine pancrease; Himedia, Mumbai, India; and amyloglucosidase from Sigma Aldrich, USA. The glucose oxidase-peroxidase diagnostic kit (GOD-POD) was procured from Span Diagnostics, Surat, India. All chemicals, reagents, and solvents used in the present study were of analytical grade and obtained from reputed companies.

## Starch Isolation

Pearl millet was pulverized into whole flour and sieved to remove excess bran. The semi-refined flour was soaked overnight in distilled water containing 0.01 g/100 g sodium azide to inhibit microbial growth. The soaked flour was screened through 60 and 150 mesh British standard sieves. The process was repeated until no more starch could be separated. The slurry obtained was washed several times with distilled water and centrifuged. The upper layer (protein) of the residue was removed with a spatula and discarded. The pH of the slurry was adjusted to 9.5 with diluted NAOH (0.1 N), stirred for 15 min, and washed several times with distilled water to remove alkali. The lower layer (starch) was suspended in distilled water and stirred for 5 h followed by centrifugation and washed until a neutral pH was reached. This step helps in complete removal of proteins and was repeated twice. The crude starch was purified by suspending in NaCl (0.1 N): Toluene (1:1) and stirred for 3 h, followed by centrifugation and washing several times with distilled water. Alcohol was added to the starch and stirred for 3 h followed by centrifugation. This process was repeated with acetone.[13] The white prime starch obtained was air dried and stored in air-tight polythene bags for further analysis.

## Physico-Chemical Properties of Starch

Quantitative estimations of moisture (AOAC: 925.10), fat (AOAC: 2003.05), protein (AOAC: 960.52; Nx6.25), and ash (AOAC: 923.03) were performed by the standard AOAC methods.[14]

### *Amylose content*

Total (TAM), soluble (SAM), and insoluble (IAM) amylose content of the flour and isolated starch from pearl millet was determined according to the method of Sowbhagya et al.[15] Determinations were carried out in triplicates. Potato amylose (Type III, Himedia, Mumbai, India) was used as a standard. Absorbance was measured at 630 nm in a semi Autoanalyzer (Span Autochem – 2011). Insoluble amylose content was calculated by subtracting the SAM content from TAM value (IAM = TAM – SAM).

### *Functional properties*

The bulk density (BD) was determined by filling the flour/starch into 10 mL measuring cylinder and gently tapping on a cloth. The values were recorded and BD was expressed as mL/g. Water and oil holding capacities were determined by the centrifuge method according to the method of Sosulki.[16] Flour/starch (1 g) was placed in a 50 mL centrifuge tubes, distilled water/oil (30 mL) was added to each tube and the contents were mixed well (30 s) using a glass rod. The tubes were allowed to stand for 10 min; an additional seven mixings were made with a 10 min rest period following each mixing. The suspensions were centrifuged at 2300 rpm for 25 min, the supernatant was decanted, the tubes were drained and dried in the oven at 50°C for 25 min cooled in a desiccator and weighed.

#### *Water solubility and swelling power (SP)*

SP and solubility was determined by the centrifuge method.[16] Flour/starch was placed in a 50 mL centrifuge tube, distilled water (30 mL) was added, mixed well, and heated at 55, 65, 75, 85, and 95°C, respectively, in a water bath with intermittent stirring for 30 min. Centrifuged at 2000 rpm for 20 min and the supernatant was decanted and evaporated on a steam bath to obtain dissolved solids. The sediment flour was weighed to obtain the weights of the swollen flour particles. Water solubility was expressed as grams of soluble solids per gram of flour (db) and SP was expressed as grams of hydrated residual solids (or gel) per gram of flour (db).

### X-Ray Diffraction

The flour and isolated starch from pearl millet, were packed in rectangular glass crucibles and exposed to x-ray beam (8 keV) generated by a x-ray diffractometer (MiniFlex –II, desktop x-ray diffractometer, Japan) equipped with a θ-θ goniometer at 25 mA and 30 kV, with Cu kα filtered radiation. The scanning range for 2θ was set to 6–45° to cover all the significant diffraction peaks of sample crystallites with a scan speed of 3°/min.

### Nutritionally Important Starch Fractions

#### *Gelatinization*

Pearl millet flour and isolated starch of both cultivars—K and MRB, respectively, were mixed with distilled water (sample to water ratio of 1:5). The mixture was stirred on a hot plate until it turned into a colorless gel. The gelatinized samples were analyzed for moisture content[10] and nutritionally important starch fractions. The values for nutritionally important starch fractions are expressed on an as—eaten basis.

Nutritionally important starch fractions such as total starch and different starch fractions—RDS (rapidly available glucose), RS (resistant starch), and RAG (rapidly available starch) were measured by the methods of Englyst et al.[10] (Fig. 2). Various starch fractions were measured in the gelatinized samples by incubating with Invertase (Himedia, Mumbai, India) to hydrolyze sucrose, pancreatin (from porcine pancrease, Himedia, Mumbai, India), amyloglucosidase (Sigma Aldrich, USA), and guar gum (to standardize the viscosity of the incubation mixture) and kept at 37°C in capped tubes immersed in a shaker water bath. A value for RAG was obtained by drawing 0.5 mL of the reaction mixture after 20 min of incubation and placed in 2 mL of 66% ethanol to terminate the enzyme reaction. After centrifugation at 3000 g for 5 min, the glucose content in the supernatant was measured using glucose oxidase peroxidase assay. A second measurement ($G_{120}$) was obtained as glucose released after the further 100 min incubation. A third measurement (total glucose [TG]) was obtained by incubating the sample in the boiling water and treatment with 7M KOH at 0°C, followed by complete enzymatic hydrolysis with amyloglucosidase (Sigma Aldrich, USA). RS was measured as the starch remained unhydrolyzed after 120 min incubation. Free glucose (FG) was also determined by treating the sample with acetate buffer and placing the tube in water bath at 100°C for 30 min. simultaneous tests were run in a similar manner with glucose standard. A blank tube containing buffer, glass balls, and guar gum was also included to correct for the glucose present amyloglucosidase solution. Glucose was determined in all the samples using glucose oxidase-peroxidase diagnostic kit (Agappe diagnostics, Kerala, India).

### Statistical Analysis

The data was subjected to analysis of variance (ANOVA) test and the differences between the means were compared for their significance ($p < 0.05$) using SPSS software v.17.

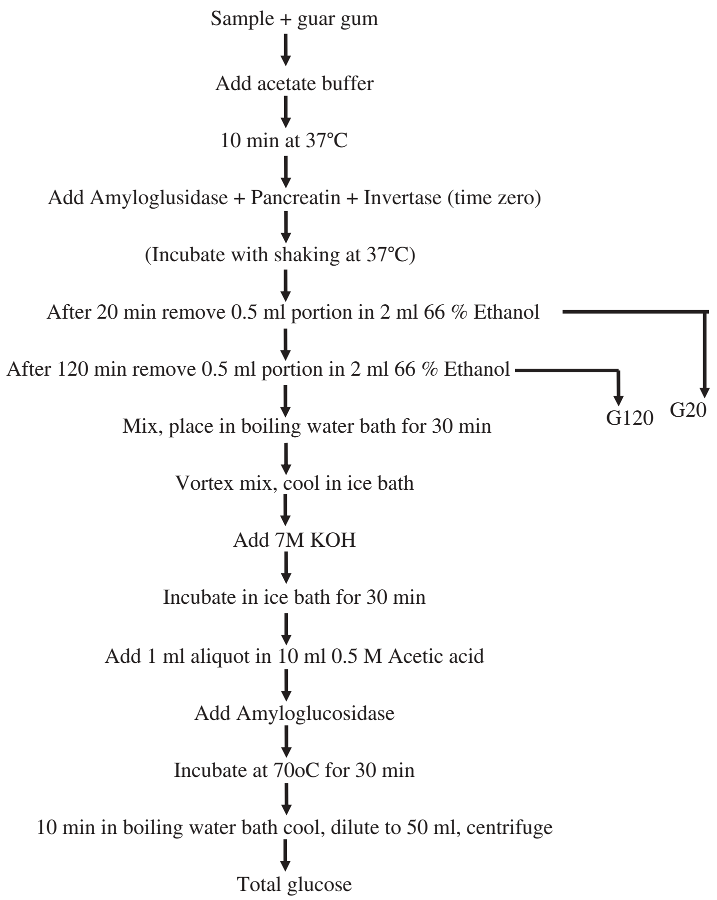


FIGURE 2 Summary of the analytical strategy for measurement of starch fractions.

## RESULTS AND DISCUSSION

### Chemical Composition of Corn and Pearl Millet Starches (g/100 g)

The data on the yield of starch from two pearl millet cultivars is presented in Table 1. As observed in the present investigation, the starch isolated from MRB (39.4 g/100 g) and K (34.5 g/100 g) was lower than the reported values (55 g/100 g).[9,8,17] These variations could be due to isolation and purification methods employed by the researcher. Low yield of starch was also reported for sorghum cultivars ranging from 27–30 g/100 g.[18] The isolated starch obtained in the present study, was pure white in color and matched with that of corn starch indicating its purity. The moisture content of pearl millet flour (about 10 g/100 g) and corn starch (9.62 g/100 g) was lower than that of starch isolated from pearl millet cultivars (about 12 g/100 g). The reported moisture content for pearl millet starch was 10.18 g/100 g while for Cocoyam starch, moisture was in the range of 9.4 to 17.3 g/100 g which was considered to be within the acceptable range and beneficial in terms of shelf life and keeping quality of the starches.[17,19] The non-starch components, such as proteins, fat, and ash, that were significantly higher in the flour were found in traces in the isolated

TABLE 1
Chemical composition of flour and isolated starch

| *Sample* | *Yield*† | *Moisture* | *Protein*£ | *Fat* | *Ash* |
|---|---|---|---|---|---|
| **CS** | – | $9.62^{a} \pm 0.14$ | $0.19^{a} \pm 0.01$ | $0.00^{a} \pm 0.00$ | $0.10^{a} \pm 0.00$ |
| **KF** | – | $10.10^{a} \pm 1.80$ | $9.30^{c} \pm 1.15$ | $4.80^{c} \pm 0.64$ | $2.00^{c} \pm 0.35$ |
| **KS** | $34.50^{a} \pm 0.5$ | $12.81^{b} \pm 0.20$ | $0.55^{b} \pm 0.01$ | $0.37^{b} \pm 0.05$ | $0.10^{a} \pm 0.00$ |
| **MRBF** | – | $9.60^{a} \pm 0.82$ | $10.20^{d} \pm 0.38$ | $5.40^{d} \pm 0.22$ | $1.50^{b} \pm 0.06$ |
| **MRBS** | $39.40^{b} \pm 0.4$ | $12.47^{b} \pm 0.20$ | $0.53^{b} \pm 0.01$ | $0.38^{b} \pm 0.02$ | $0.10^{a} \pm 0.00$ |

g/100 g dry basis;
†The value represents the mean of three determinations on whole flour basis;
£Nitrogen × 6.25;
Means followed by different letters (a, b, c) in the same column differ significantly ($p \leq 0.05$);
CS: corn starch, KF: Kalukombu flour, KS: Kalukombu starch, MRBF: Maharashtra Rabi Bajra flour, MRBS: Maharashtra Rabi Bajra starch.

starch and were significantly lower than corn starch (control). These values are in accordance with other researchers.[7,8,17]

## Amylose Content (g/100 g)

TAM, corresponding SAM, and IAM of pearl millet flour and isolated starch are shown in Table 2. SAM represents the swollen granules and fragments of gelatinized granules that remain soluble while IAM corresponds to amylose and/or amylopectin long external chains, which has not leached out from the granule and capable of forming complexes with iodine when treated with chaotropic solvents such as NaOH.[20] Amylose content varies depending on the variety and method used. TAM content of pearl millet flour was 3.47 and 2.89 g/100 g and isolated starch was 3.96 and 4.96 g/100 g for K and MRB, respectively, which were lower than that of corn starch (CS). Based on the amylose content, starch can be classified as non-waxy (17–31.9 g/100 g), low-amylose (7.8–16 g/100 g), and waxy types (0–3.5 g/100 g). The starch of the non-waxy type contains both amylopectin and amylose molecules, whereas the waxy type contains only amylopectin and no or very little amylose.[21,22] Accordingly, in the present study, starch from the two cultivars (K and MRB) as well as corn starch (control) could be classified as waxy type suggesting that these cultivars contained very little amylose. Higher amylose content has been reported by other researchers for pearl millet starch.[23,24] The SAM content in starches from pearl millet and corn were higher compared to the flour. Kalukombu starch (KS) exhibited lower IAM while CS showed the highest. CS showed higher amylose and/or amylopectin that remained inside the swollen starch granule. Amylose content influences the gelatinization temperature; high amylose content restricts the

TABLE 2
Amylose content of pearl millet flour and starches (g/100 g)

| *Sample* | *SAM* | *IAM* | *TAM* |
|---|---|---|---|
| CS | $2.64^{c} \pm 0.06$ | $4.17^{e} \pm 0.12$ | $6.81^{e} \pm 0.06$ |
| KF | $1.06^{a} \pm 0.07$ | $2.41^{d} \pm 0.06$ | $3.47^{b} \pm 0.05$ |
| KS | $3.01^{d} \pm 0.05$ | $0.95^{a} \pm 0.09$ | $3.96^{c} \pm 0.10$ |
| MRBF | $1.72^{b} \pm 0.03$ | $1.17^{b} \pm 0.05$ | $2.89^{a} \pm 0.07$ |
| MRBS | $2.93^{d} \pm 0.02$ | $2.03^{c} \pm 0.06$ | $4.96^{d} \pm 0.16$ |

SAM: Soluble amylose, IAM: Insoluble amylose, and TAM: Total amylose.

granule swelling thereby increasing the gelatinization temperature.[24] In the present study, since pearl millet and corn were of waxy type, the starch showed low gelatinization temperature. This was evident in the swelling and solubility patterns, where swollen starch granule of pearl millet and corn disintegrated at lower temperature (65–70°C) resulting in a thick paste.

## Water Holding Capacity (WHC)/Oil Holding Capacity (OHC) and BD of Pearl Millet and Corn Starches

The WHC is an important functional attribute of all flours and starches used in food preparations such as custard, dough, etc. In the two flour samples it was 1.68 and 1.65 g/g (Table 3), while in the corresponding isolated starches it was found to be lower than the flours (0.48 and 0.44 g/g for K and MRB, respectively), and were comparable with corn starch (0.41 g/g). The ability of food materials to absorb water is sometimes attributed to its protein and fiber content.[25] The low WHC in the isolated starches could be attributed to low protein content and possible absence of fiber. OHC is useful in structure interaction of food particularly in flavor retention, improvement of palatability, and extension of shelf life in bakery and meat products.[22] OHC of the K and MRB flours was 1.38 and 1.18 g/g, respectively, while isolated starches exhibited a lower OHC of 0.73 and 1.27 g/g, respectively. The starch from MRB showed higher oil uptake compared to the flour. A possible explanation for the increased oil uptake of starches could be due to the varietal differences and larger surface area and porosity of the particles. The compactness of the flour could have resulted in low porosity that restricted oil uptake, compared to starch that had larger surface area which increased oil uptake.[26] The OHC of pearl millet starches were lower than corn starch (1.90 g/g). The BD is influenced by the moisture content of the grain. The flour of pearl millet had a moisture content of about 10 g/100 g and exhibited a BD of 0.88 and 0.95 mL/g for K and MRB, respectively. Whereas the respective pearl millet starches contained about 12 g/100 g moisture and exhibited a higher BD of 1.90 and 1.85 mL/g. In the present study, BD increased with the moisture content. Similar findings were reported for coffee and pistachios, where BD increased linearly with increase in grain moisture content.[27,28]

## SP and Solubility of Flour and Starch of Pearl Millet Cultivars

SP and solubility of starch indicates non covalent bonding between molecules within a starch molecule.[19] It can be used to assess the extent of interaction between starch chains, within the amorphous and crystalline domains of the starch granules.[29] Swelling and solubility of pearl millet flour and starches were determined over a range of temperatures (55–95°C). SP of flour ranged from about 2.02 – 14.75 g/g and for isolated starch, it ranged from 2.12 – 19.65 g/g (Fig. 3). It increased linearly with temperature (55 – 95°C) indicating that the rate of water absorption and

TABLE 3
Functional properties of flour and isolated starch (mL/g)

| *Sample* | *WHC* | *OHC* | *BD* |
|---|---|---|---|
| **CS** | $0.41^{a} \pm 0.01$ | $1.90^{d} \pm 0.08$ | $1.85^{b} \pm 0.05$ |
| **KF** | $1.68^{b} \pm 0.10$ | $1.38^{c} \pm 0.10$ | $0.88^{a} \pm 0.11$ |
| **KS** | $0.48^{a} \pm 0.01$ | $0.73^{a} \pm 0.08$ | $1.90^{c} \pm 0.05$ |
| **MRBF** | $1.65^{b} \pm 0.20$ | $1.18^{b} \pm 0.00$ | $0.95^{a} \pm 0.05$ |
| **MRBS** | $0.44^{a} \pm 0.03$ | $1.27^{c} \pm 0.09$ | $1.90^{c} \pm 0.05$ |

WHC: Water holding capacity, OHC: Oil holding capacity, and BD: Bulk density;
Means followed by different letters (a, b, c) in the same column differ significantly ($p \leq 0.05$).

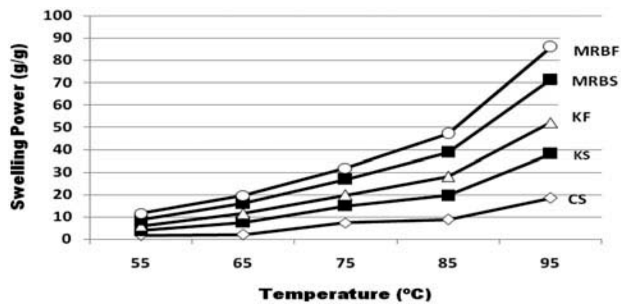


FIGURE 3 Swelling power of flour and isolated starch (g/g); CS: corn starch, KF: Kalukombu flour, KS: Kalukombu starch, MRBF: Maharashtra Rabi Bajra flour, and MRBS: Maharashtra Rabi Bajra starch.

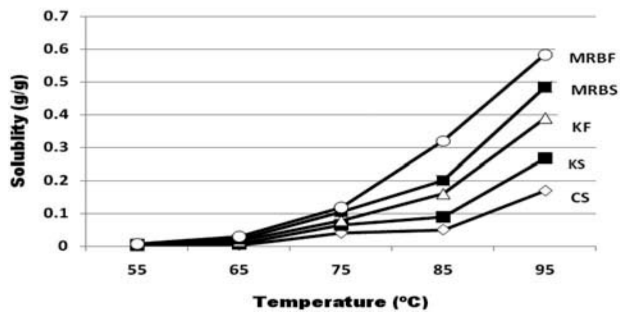


FIGURE 4 Solubility of whole flour and starches isolated from pearl millet; CS: corn starch, KF: Kalukombu flour, KS: Kalukombu starch, MRBF: Maharashtra Rabi Bajra flour, and MRBS: Maharashtra Rabi Bajra starch.

swelling of pearl millet flour and isolated starches was temperature dependent. The solubility pattern was determined in combination with SP, which ranged from 0.001 – 0.125 g/g for flours and 0.00 – 0.017 g/g for the isolated starches (Fig. 4). Solubility was linearly associated with temperature increase, perhaps due to weakening of intragranular bonds leading to amylase leaching at a higher temperature.[30] These observations are similar those reported on pearl millet starch where lower solubility was observed below 75°C and thereafter increased from 80°C onward.[7] This phenomenon is related to a two stage relaxation of bonding forces within the starch granules during swelling which showed lower values during first stage of relaxation and higher values in the second stage. Low amylose is associated with higher starch granule disintegration. Since pearl millet and corn were of waxy or low amylose type, it resulted in the fragility of swollen starch granules that disintegrated easily at temperatures above 65°C.

## X-Ray Diffractograms

Starch is semi-crystalline in nature which can be identified through characteristic x-ray diffraction patterns. In the x-ray diffractogram, the sharp peaks are associated with the crystalline region while the diffused peaks with the amorphous region of the samples.[31] Corn starch (control) exhibited singlet and sharper peaks at 2θ values of 15 and 23º and a double or diffused peak at 2θ of 17 and 18º which is a characteristic of A-type crystallinity (Fig. 5). Similarly, K and MRB also showed sharp peaks at 2θ values 15 and 23º and a diffused peak at 2θ of 17 and 18º. The A-type is characteristic of starches that are of cereal origin. The diffraction pattern of corn and pearl millet

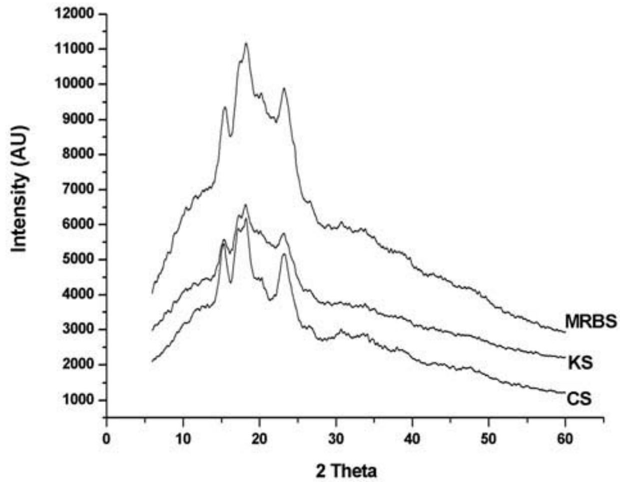


FIGURE 5 X-ray diffractograms of pearl millet and corn starches.

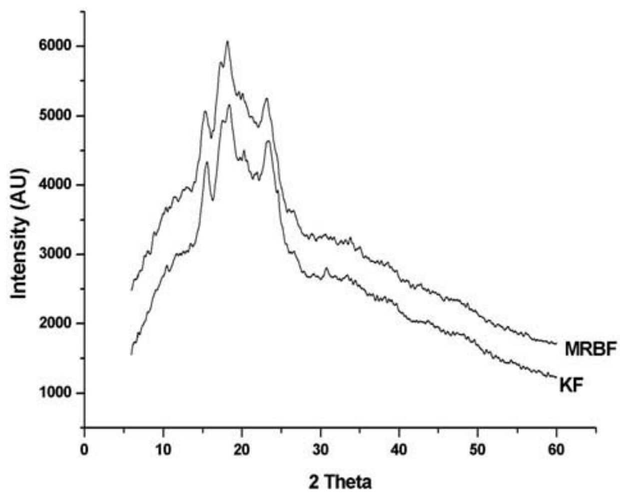


FIGURE 6 X-ray diffractograms of pearl millet flours.

starches were similar to any other unprocessed cereal starch indicating its semi-crystalline nature.-[7,32] Therefore, in the present study, all the three starches exhibited a semi-crystalline structure of type A. The diffractograms of K and MRB flours were (Fig. 6) similar to their isolated starches. However, the intensity of the peaks for the starch was comparatively higher than that of the respective flour samples. The x-ray diffractograms mainly represent the type of the starch present in the sample. In addition to the starch the whole flour, also contains other components such as protein, dietary fiber, and fat. The presence of these components might have interfered with the diffraction pattern of the samples. Hence, slightly sharper peaks for the starch samples were obtained.

## Nutritionally Important Starch Fractions

Nutritionally important starch fractions analyzed in pearl millet and corn starch as well as pearl millet flours in both cultivars is presented in Table 4. Gelatinization is an important step in food processing. This produces an edible product, increases its nutritive value, and generates desirable flavor and texture. Hence, all the samples were gelatinized prior to analysis and expressed on an as-eaten basis. The total starch content ranged from 20.2 g/100 g (corn starch) to 23 g/100 g (MRB starch). The starch from pearl millet and corn exhibited similar total starch (TS) values. Nutritionally, starch is divided into RDS, SDS, and RS. Flour from pearl millet showed RDS, SDS, and RS values of 10.2 and 11.4 g/100 g, 6 and 7.6 g/100 g, and 5.1 and 4.0 g/100 g for K and MRB, respectively. The amount of RS depends on the amylose-lipid complexes and presence of other materials in the food matrix (sugar, protein, etc.).[33,34] RS has a direct impact on glycaemic response, prevents constipation, and increases fecal bulk. The RS values for flour and starch in both pearl millet cultivars ranged from 1.4–5.1 g/100 g. Low levels of fat, fiber, or proteins may have lead to lower RS values in the isolated starch. However, RS content of 10–20 g/100 g is required to make a substantial difference to the physiological properties of the food.[33,34] Flour and isolated starch of both cultivars showed A-type crystalline structure when examined with x-ray diffraction (Figs. 4 and 5). A- and B-type crystalline pattern are known to influence the enzyme digestion. A-type crystalline structure is easily hydrolyzed by enzymes, resulting in higher RDS, SDS, and low RS than the B-type starch The lower RS content of these gelatinized starches was apparently due to the A-type starch and due to the absence of elimination of structural obstruction to amylase hydrolysis during the process of starch isolation.[35] Furthermore, during the process of gelatinization, the crystalline structure gets disrupted and increases the accessibility of glucose chains to amylolytic enzymes.[36]

Starch digestion index (SDI) is a measure of the relative rate of starch digestion. In the present investigation, SDI ranged from 49% (K flour) to 57% (MRB starch, Table 5). Similar values were reported for freshly cooked spaghetti (52), millet (55), and lentils (44). SDI was comparatively low in the isolated pearl millet starch and corn (control) may be due to the physical form of the millet based starches are partly inaccessible to the digestive enzymes.[13] The simple *in vitro* measurement of RAG is of physiologic relevance and could serve as a tool for investigating the importance of the amount, type, and form of dietary carbohydrates for health.[37] RAG (Table 5) values represent the amount of glucose that is rapidly available for absorption after a meal. It is a good indicator of blood glucose and insulin response as it includes RDS and FG. In the present study, RAG ranged from 11.3 (corn) to 13.6 (MRB). Similar RAG values were reported for instant potatoes, millet, and spaghetti (approximately 13 g/100 g).[10] Low RAG values could be attributed to the fact that time for gelatinization was too short for complete gelatinization of starch molecules and due to the dense matrix which hindered enzymatic hydrolysis of starch.

TABLE 4
Total starch (TS) and its nutritionally IMPORTANT FRACTIONS (g/100 g)

| *Sample* | *Moisture (g/100g)* | *TS* | *RDS* | *SDS* | *RS* |
|---|---|---|---|---|---|
| **CS** | $73.0^{c} \pm 0.00$ | $20.2^{a} \pm 0.25$ | $10.2^{a} \pm 0.27$ | $7.2^{b} \pm 0.35$ | $2.8^{b} \pm 0.52$ |
| **KF** | $86.8^{e} \pm 0.20$ | $21.3^{ab} \pm 0.69$ | $10.2^{a} \pm 0.29$ | $6.0^{a} \pm 0.12$ | $5.1^{d} \pm 0.81$ |
| **KS** | $64.6^{b} \pm 2.73$ | $22.5^{b} \pm 0.80$ | $11.3^{b} \pm 0.31$ | $9.0^{c} \pm 0.29$ | $2.2^{ab} \pm 0.23$ |
| **MRBF** | $85.9^{d} \pm 0.11$ | $23.0^{c} \pm 0.35$ | $11.4^{b} \pm 0.63$ | $7.6^{b} \pm 0.48$ | $4.0^{c} \pm 0.90$ |
| **MRBS** | $52.7^{a} \pm 0.00$ | $21.5^{ab} \pm 0.00$ | $12.2^{c} \pm 0.18$ | $7.9^{b} \pm 0.34$ | $1.4^{a} \pm 0.19$ |

g/100 g fresh basis;
Means followed by different letters (a, b, c) in the same column differ significantly ($p \leq 0.05$);
RDS: Rapidly digestible starch, SDS: Slowly digestible starch, and RS: Resistant starch.

TABLE 5
Rapidly available glucose (RAG) and starch digestibility index (SDI) of pearl millet flour and isolated starch

| *Sample* | *SDI (%)* | *RAG (g/100 g)* |
|---|---|---|
| **CS** | $50^{a} \pm 1.00$ | $11.3^{a} \pm 0.30$ |
| **KF** | $49^{b} \pm 2.06$ | $13.7^{c} \pm 0.16$ |
| **KS** | $50^{a} \pm 0.45$ | $12.5^{b} \pm 0.34$ |
| **MRBF** | $50^{a} \pm 2.30$ | $13.2^{c} \pm 0.70$ |
| **MRBS** | $57^{c} \pm 0.84$ | $13.6^{c} \pm 0.20$ |

g/100 g fresh basis;
Means followed by different letters (a, b, c) in the same column differ significantly ($p \leq 0.05$).

## CONCLUSION

Pearl millet being less expensive compared to cereals like rice or corn, has greater potential in food applications. It could be successfully used as a low-cost replacement for corn starch, especially in countries where pearl millet is a staple crop. In view of this, starch was isolated from two pearl millet cultivars—K and MRB—and explored for its physico-chemical properties, nutritionally important starch fractions and x-ray diffraction in comparison with corn starch. Starches isolated from pearl millet cultivars K and MRB contained significantly low levels of non-starch components indicating its purity. Starches from MRB had higher amylose, OHC, and RDS, while K exhibited higher SDS content. Compared to corn starch (commercial starch source) pearl millet exhibited higher moisture, protein, and fat content; however, these values were within the acceptable range. Higher SP and solubility at >65ºC indicates its usefulness as thickeners. The nutritional attributes of starch such as high RDS and low RS indicates its use in easily digestible formulations for infants, geriatrics, and convalescents.

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